AD_____

Award Number: W81XWH-05-D-0001

TITLE: Operate a Chemical Surety Program and Studies Supporting the Medical Chemical Defense Research Program

PROGRAM MANAGER: James A. Blank, Ph.D., D.A.B.T.
PRINCIPAL INVESTIGATOR: Michael C. Babin, D.V.M., Ph.D.

CONTRACTING ORGANIZATION: Battelle Memorial Institute
Biomedical Research Center
Columbus, Ohio 43201-2696

REPORT DATE: May 2010

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5102

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01-06-2010	Annual Report	2 May 2009 – 1 May 2010

4. TITLE AND SUBTITLE
Operate a Chemical Surety Program and Studies Supporting the Medical Chemical Defense Research Program

5a. CONTRACT NUMBER
W81XWH-05-D-0001

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Michael C. Babin, D.V.M., Ph. D.

E-Mail: BabinM@battelle.org

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Battelle Memorial Institute

Columbus, OH 43201-2696
E-Mail: BabinM@battelle.org

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, MD 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
For Task Order (TO) 1, the facilities were maintained and operated in compliance with government regulations; chemical agent (CA) inventories were maintained; and 34 TEPs for task orders were prepared in addition to TO 0001. Of the 34 TEPs requested, 27 have been awarded, 7 have been cancelled, and none are pending award. These TEPs encompass areas dealing with toxicity testing, sulfur mustard skin and eye injury, thermal burns, skin penetration of agents, synthesis, pharmacokinetics, efficacy testing, seizure control, bioscavengers, toxicology testing and test kit evaluations. In addition, TO 12 provides on-site study support to the USAMRICD Collaborative Research Facility.

15. SUBJECT TERMS
Medical countermeasures, animal models, protection, genotoxicity, reactivator, MMB-4, treatment, HD, SERPACWA, penetration cell, NTA, toxicology, wound healing, GLP, microarray, thermal injury, nerve agent, aerosol, midazolam, enzyme test kit, bioscavenger

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU	33	**19b. TELEPHONE NUMBER** *(include area code)*

MAY 2010
ANNUAL REPORT ON CONTRACT
W81XWH-05-D-0001

TABLE OF CONTENTS

Page

INTRODUCTION 1

BODY 1

Task Order 0001 – Operate a Chemical Surety Program and Provide Management Support 1

Task Order 0010 – Efficacy of Anti-Inflammatory Therapy 2

Task Order 0011 – Definitive Animal Studies with Midazolam 9

Task Order 0012 – Studies Supporting the USAMRICD CRF 11

Task Order 0013 – Evaluation of a BioScavenger in a Nonhuman Primate Model of Chemical Agent Poisoning 13

Task Order 0018 – Collection of Tissue Samples from Testing Compounds Againts Vapor-Induced Sulfur Mustard Injury in the Hairless Mouse Model 14

Task Order 0019 – Treatment of Sulfur Mustard Exposure using the Rabbit Eye Model 15

Task Order 0020 – Personnel Decon: Live Chemical Agents Protocol 17

Task Order 0024 – Post-Exposure Medical Countermeasures Against Inhalation Toxicity of OP 17

Task Order 0028 – Comparative Efficacy of Intravenous Hydroxocobalamin and Sodium Thiosulfate/Sodium Nitrite Administrations Following Intravenous Poisoning with Potassium Cyanide in Adult Male Beagle Dogs 18

Task Order 0029 – Characterization of Agent Mechanisms of Toxicity and Toxicokinetic Properties 20

Task Order 0031 – Static Temperature Testing of Reactive Skin Decontamination Lotion (RSDL) with a Time Temperature Indicator (TTI) 21

Task Order 0032 – Testing Therapeutic Countermeasures against Ocular Injuries Following Sulfur Mustard Exposure in Rabbits..... 22

Task Order 0033 – Development of a Process to Produce Large Amounts of the Oxime HLo-7 23

Task Execution Plan 34 – Temperature Testing of Pyridostigmine Bromide (PB)..... 23

KEY RESEARCH ACCOMPLISHMENTS.....24

REPORTABLE OUTCOMES.....28

CONCLUSION.....28

REFERENCES 28

APPENDICES 28

SUPPORTING DATA.....28

MAY 2010
ANNUAL REPORT ON CONTRACT
W81XWH-05-D-0001

Operate a Chemical Surety Program and Studies Supporting the Medical Chemical Defense Research Program

INTRODUCTION

The Department of Defense (DoD) Team consisting of the Defense Threat Reduction Agency (DTRA), Chemical Biological Medical Systems (CBMS), a division of the Joint Program Executive Office for Chemical and Biological Defense (JPEO-CBD), and the U.S. Army Medical Research and Materiel Command (USAMRMC) has a mission-critical goal to improve military effectiveness and survivability of the service member in a chemically contaminated environment. Battelle's Biomedical Research Center (BBRC) formerly named the Medical Research and Evaluation facility (MREF), support this goal.

The BBRC answers and validates basic, applied, and developmental biomedical questions critical to providing improved medical countermeasures against chemical agents (CA), toxins and emerging threats. This research, development, testing, and evaluation (RDT&E) facility uses animal models, alternatives to animal models, and complex laboratory procedures utilizing CA and other hazardous chemicals. The facility provides ample space for studies in multiple animal species, *in vitro* models, including isolated organ systems, cell culture, analytical, medicinal, and synthetic chemistry procedures. Studies involve parenteral, oral, or topical administration of candidate prophylaxes, pretreatment compounds, protective and/or decontamination materials in conjunction with percutaneous, parenteral, or inhalation challenge with threat agents utilizing *in vivo* model systems. The facility meets all safety and surety requirements for storage, handling, use, and disposal of CA, RDT&E dilute solutions of CA and other hazardous materials. The BBRC has the demonstrated and documented ability to conduct research and testing that satisfies the requirements of Good Laboratory Practices (GLP) regulations and ISO 9001 standards.

BODY

Task Order 0001 – Operate a Chemical Surety Program and Provide Management Support

Program Manager: Dr. James A. Blank

Government POC(s): Dr. David Lenz (DTRA/CBMS/MRMC COR)

Period of Performance: May 2, 2005 to November 1, 2010
(Pending extension to November 1, 2011)

The BBRC's laboratories and facilities were maintained and operated in compliance with government regulations. Major contract activities performed include: conducting inventories of CA and maintaining usage reports, preparing 7 Test Execution Plans (TEP) for task orders with two TEPs involving extensive coordination with Government principle investigators and project managers. Program management and Battelle investigators conducted frequent telephone, email and on-site discussions with Government Principle Investigators and Program Leadership to support various Medical Chemical Defense Program projects. Numerous scientific discussions were held with Department of Defense Team representatives for the development of projected tasks. The BBRC successfully passed all inspections or certifications by the U.S. Department of Agriculture, Ohio Environmental Protection Agency, Madison County (OH)

Health Department, Battelle's Institutional Animal Care and Use Committee (IACUC), ISO 9001 Registrar, and the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC International). The BBRC also provides administrative support for an onsite Contracting Officer's Representative (COR). Provided program support to meet contract reporting requirements to include reviews; quarterly, annual, and final reports; property inventory reports; and reportable events.

Task Order 0010 - Wound Healing of Cutaneous Chemical Injuries: Efficacy of Anti-inflammatory Therapy

Task Leader: Dr. Frances Reid / Ms. Robyn Kiser

Government POC(s): Dr. John Graham (MRMC-USAMRICD

Period of Performance: September 1, 2006 through April 30, 2011
(Research ends January 31, 2011)

Description of Studies:

Two protocols were written to evaluate the efficacy of steroidal anti-inflammatory compounds in treating cutaneous injuries caused by exposure to a toxic industrial chemical (TIC) or by sulfur mustard. One protocol is for a non-GLP bromine dose-ranging study, and the second protocol is for a GLP steroid efficacy study for treating bromine and sulfur mustard lesions.

1. Bromine dose-ranging protocol (Protocol 647): Twelve, Yorkshire-cross, female pigs, weighing ~11 kg were used to determine that 600 µl of undiluted bromine applied to the skin (4 sites on the ventral abdomen with each site exposed for a different exposure length) of weanling swine for 45 s caused a superficial dermal injury and an 8 min exposure caused a deep dermal injury. Twelve animals were used to evaluate the toxicogenomics of bromine. The sites were evaluated after 24 and 48 h and included clinical observations, digital photographs, size measurements, modified Draize scoring, reflectance colorimetry, evaporimetry, and infrared thermography. Histopathologic evaluation of skin collected at 48 h post-exposure included wound severity, wound depth, and percent of the total area involved.

2. and 3. GLP steroid efficacy study (Protocol 648): Thirty-six female, Yorkshire-cross pigs for each agent/TIC were randomly assigned to six experimental groups, corresponding to all combinations of the three treatment compounds and two pre-planned sacrifice days, either 3 or 10 days after exposure. Animals were exposed to approximately 400 microliters (µl) of undiluted HD for either 8 min or 30 min, or 600 µl of undiluted BR for either 0.75 min or 8 min on each of four ventral abdominal sites. Each side of the ventral midline represented either a superficial dermal injury (SD, 8 min for HD or 0.75 for BR) or a deep dermal injury (DD, 30 min for HD or 8 min for BR). Application of the steroid to one site of each depth of injury began at one hour after the end of exposure of that site, and was repeated every four hours for a total of four daily treatments over a period of three days. The untreated sites served as within-animal control sites. Assessments were conducted on Study Days 0 (evaporimetry only), 2, 3, and 10 (for 10 day survivor animals). Assessments included digital photographs, lesion size measurements, modified Draize Scoring, and non-invasive bioengineering techniques, i.e. reflectance colorimetry, infrared imagery, and transepidermal water loss (TEWL). Tissues were fixed in 10% neutral buffered

formalin, embedded in paraffin, cut on a microtome, mounted on a slide and stained with hematoxylin and eosin (H&E) for histopathology.

Task 10 was expanded to design, fabricate, and perform dose ranging studies using a bromine vapor exposure system to produce superficial and deep dermal injuries on the ventral abdomen of weanling swine. A GLP efficacy study will be conducted to test three NSAIDs with clobetasol applied topically to HD and bromine vapor injuries.

4. Bromine vapor system design and fabrication, and dose-ranging protocol (Protocol 772): The bromine vapor exposure system was designed, fabricated, and characterized. Twelve, Yorkshire-cross, female pigs, weighing ~11 kg were used to determine the length of time for bromine vapor to contact the skin (4 sites on the ventral abdomen with each site exposed for a different exposure length) to produce a superficial dermal injury and a deep dermal injury. The sites were evaluated after 24 and 48 h and included clinical observations, digital photographs, size measurements, modified Draize scoring, reflectance colorimetry, evaporimetry, and infrared thermography. Histopathologic evaluation of skin collected at 48 h post-exposure included wound severity, wound depth, and percent of the total area involved. Nine animals were used to evaluate the toxicogenomics of bromine vapor with 3 of these animals from the last 3 animals exposed under the dose ranging study and for 6 h, 48 h, and 7 days.

5. and 6. GLP NSAID with steroid efficacy study (Protocol 773): Thirty-six female, Yorkshire-cross pigs for each agent/TIC were randomly assigned to six experimental groups, corresponding to all combinations of the three NSAID (Capsaicin, octylhomovanilamide and 4-methyl-2-mercaptopyridine-1-oxide) with Clobetasol treatment compounds and two pre-planned sacrifice days, either 3 or 10 days after exposure. Animals were exposed to approximately 400 microliters (µl) of undiluted HD for either 8 min or 30 min, and as determined from the bromine vapor dose ranging study on each of four ventral abdominal sites. Each side of the ventral midline represented either a superficial dermal injury (SD, 8 min for HD or to be determined) or a deep dermal injury (DD, 30 min for HD or to be determined). Application of the NSAID with steroid to one site of each depth of injury began at one hour after the end of exposure of that site, and was repeated every four hours for a total of four daily treatments over a period of three days. The untreated sites served as within-animal control sites. Assessments were conducted on Study Days 0 (evaporimetry only), 2, 3, and 10 (for 10 day survivor animals). Assessments included digital photographs, lesion size measurements, modified Draize Scoring, and non-invasive bioengineering techniques, i.e. reflectance colorimetry, infrared imagery, and transepidermal water loss (TEWL). Tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, cut on a microtome, mounted on a slide and stained with hematoxylin and eosin (H&E) for histopathology.

Task 10 was expanded to design, fabricate, and perform dose ranging studies using a chlorine vapor exposure system to produce superficial and deep dermal injuries on the ventral abdomen of weanling swine. In addition, a non-GLP efficacy study will be conducted to optimize a treatment regimen using either Capsaicin or Diclofenac with clobetasol that will ameliorate the effects of HD and to select the best regimen to use in a future GLP study.

7. Determine a chlorine vapor concentration and exposure time that creates superficial dermal and deep dermal injuries in the weanling pig, while also collecting tissues for genomics evaluation of potential biomarkers (sub title: Vapor Chamber Fabrication, Characterization and Dose Ranging with Toxicogenomics using Vapor Chlorine Exposure, Protocol 910);

8. Select the most efficacious steroidal and non-steroid anti-inflammatory (NSAID) drug for treating superficial dermal and deep dermal sulfur mustard-induced injuries and determine the most efficacious treatment regimen (subtitle: Treatment Regimen of a Steroidal and Non-steroidal Anti-Inflammatories Against Sulfur Mustard, Protocol 911).

Task 10 was expanded to conduct a GLP study to test the diclofenac with clobetasol treatment of 4 times a day for 5 days to ameliorate the effects of sulfur mustard superficial and deep dermal injuries on the ventral abdomen of weanling swine.

9. A definitive GLP efficacy study to test the most efficacious treatment regimen of an NSAID with a steroid in treating sulfur mustard induced lesions [sub title: Definitive Treatment Regimen for Treating Sulfur Mustard Skin Lesions Using a Steroidal Anti-Inflammatory Agent in Combination with a Non-Steroidal Anti-Inflammatory Drug (NSAIDs) Protocol 1054];

10. Determine the most efficacious steroidal with non-steroid anti-inflammatory (NSAID) and/or alternate drug for treating superficial dermal and deep dermal sulfur mustard-induced injuries and determine the most efficacious treatment regimen (subtitle: Efficacy Testing of New Pharmaceutical Drugs for Treating Sulfur Mustard and Chlorine Vapor Induced Skin Lesions, Protocol 1055).

Progress:

Validation of the equipment used in the GLP portions of this study began in early February 2007 and was completed. Yearly maintenance of equipment is completed in-between studies.

1. Final Report submitted and approved. Summary: The bromine dose-ranging protocol (Protocol 647) was approved by Battelle's IACUC on December 27, 2006 and submitted to the USAMRMC Animal Care and Use Review Office (ACURO) on January 18, 2007. ACURO review questions were posed April 11th and 18th, and responses were sent April 13th and 23rd. ACURO approval was received on May 8, and the study was initiated on June 25, 2007. Twelve animals were used to determine that 600 µl of undiluted bromine applied to the skin of weanling swine for 45 s caused a superficial dermal injury and an 8 min exposure caused a deep dermal injury. A peer-review of the histopathology determined that a superficial dermal injury was not created, and that at both time points a deep dermal to full-skin-thickness injury resulted. Bromine vapor exposures may provide greater control of the depth of burn. An additional 12 animals were used in toxicogenomic studies. Four time points (6, 24 and 48 h, and 7 d) were evaluated, with data obtained for all but the 48 h time point. Data analysis indicated differences in time course but not between exposure times for depth of injury. Deep dermal injuries were created by 600 µL of liquid bromine at exposure lengths for 30 s or longer. At all exposure lengths tested (30 s to 8 min), deep dermal to full-skin-thickness lesions were created.
   Genomics: Four time points (6, 24 and 48 h, and 7 d) were evaluated, with data obtained for all but the 48 h time point. Expression analysis revealed that bromine exposure duration appeared to have less effect on the transcript changes than the sampling time. The percent transcripts changed at 24 h were similar (30%) for a 45 sec (45s-24 h) or 8 min (8m-24 h) bromine exposure; percent transcripts changed at 7 days were also similar (62%) regardless of exposure time (45 s-7 d versus 8 min-7 d). However, only 13 to 14% of the transcripts were similar when comparing samples analyzed at 24 h and 7 d. Ingenuity Pathways Analysis (IPA) revealed six common biological functions out of the top ten observed to be shared in all groups, while canonical pathway analysis

revealed 11 genes that were commonly shared among 24 significantly altered signaling pathways. Additionally, there were 11 signaling pathways in which there were no commonly-shared transcripts.

2. and 3. Final Report submitted and approved. Summary: The GLP steroid efficacy study (Protocol 648) was approved by Battelle's IACUC on February 12, 2007 and submitted to the USAMRMC ACURO on March 15, 2007. ACURO review questions were posed April 10th, and responses were sent April 23rd. ACURO approved this task on May 2, 2007, and HD exposures began in July. Bromine exposures were initiated in August and *in vivo* procedures were completed by November 2, 2007. Results indicated Clobetasol propionate had the most promise for healing HD-induced lesions. Steroid treatment of HD-induced lesions showed improved healing, particularly for superficial dermal injuries and with higher potency steroids. Steroids from three different categories of potency were not effective in ameliorating deep bromine injuries. Bromine injuries created using 45 s and 8 min exposure times were deep dermal to full-skin-thickness injuries. Superficial dermal injuries were not created in this study.

Task 10 was expanded to design, fabricate, and perform dose ranging studies using a bromine vapor exposure system to produce superficial and deep dermal injuries on the ventral abdomen of weanling swine.

4. A final report is being drafted and near completion. Summary: A bromine vapor system was designed, fabricated, characterized, and a dose-ranging protocol (Protocol 772) written and executed. The bromine vapor dose-ranging protocol (Protocol 772) was approved by Battelle's IACUC on March 11, 2008 and the USAMRMC's ACURO approval was received on April 3, 2008. Twelve animals were used to determine the concentration and exposure time of bromine vapor that will induce a superficial dermal injury and a deep dermal injury. The bromine vapor exposure system was initially designed to deliver an exposure of 100 ppm of bromine vapor up to 47,000 ppm, however, no injuries were observed. Lesions were produced with exposures between 155,000 and 174,000 ppm and histopathology reported deep dermal to full thickness injuries were developed. Seven animals were exposed to concentrations ranging from 103,000 to 110,000 ppm and histopathology reported extreme variability in depth of injury and a consistent superficial dermal injury could not be created. It was noted that the metallic parts of the system were being corroded by the bromine resulting in a black liquid substance leaking into the cups and down onto the lesion sites, possibly causing the high variability in dermal injury depth and severity. Modifications to the system were made to reduce the metal parts exposed to the bromine vapor and replace them with Teflon parts resulting in a dramatic reduction in the black liquid to where none was observed. Five pigs were exposed to approximately (~) 85,000 ppm bromine vapor for 7 min resulting in a superficial dermal depth of injury for 17 min resulting in a deep dermal injury. Six animals had one side exposed to ~85,000 ppm of bromine vapor for 7 min and the opposite side exposed for 17 min, then tissues harvested in two animals at 6 h, 48 h, and at 7 days for genomic and histopathology evaluation. The *in vivo* portion of this study was completed by the end of October 2008. The 7-min exposure time for superficial dermal bromine vapor lesions was selected for use in Protocol 773. The draft final report is being prepared.

5. and 6. A draft final report is being completed. Summary: The GLP NSAIDs with Steroid Efficacy Study (Protocol 773) was approved by Battelle's IACUC on March 26, 2008 and ACURO approval was received on April 8, 2008. The exposures began July 21, 2008 with HD. After two weeks of dosing there was an indication that the treatments were showing mild irritation to the mucous membranes of staff members and mild to severe irritation on the skin of

the animals. One treatment (4-methyl-2-mercaptopyridine-1-oxide) was demonstrating a severe reaction in tissue surrounding the lesion. Dr. Graham was notified along with Battelle's safety and nursing staff. The third week of dosing was modified and approved by Battelle's IACUC and MRMC ACURO to conduct one day of 4 treatments at current dosages within the chemical fume hood with observations and assessments as described in the protocol. The results indicated that the 4MP still caused mild injury to the animal. The study was stopped after a telephone conference was held with Dr. Graham and the Study Director. The data was assembled for review by the Study Director with the USAMRICD sponsor and his associate to assess if a dose-response affect was being observed in the pigs. Safety assessments of current procedures and health issues were assessed and a document covering PPE written by safety presented to the study director. The written document addressed appropriate PPE for various procedures associated with the study. This handout and an oral presentation with this information was presented to the staff associated with these studies and then to all technical staff by the safety officer and the study director. No further adverse affects were reported. Another 24 h study to assess the NSAIDs alone at full dose and half dose was approved by Battelle's IACUC and MRMC ACURO for conduct during the sponsors' (Dr. John Graham and Dr. William Smith) visit. The results indicated that 4MP did cause irritation to the sites (HD lesions were more severe) at both full and half dose. After much discussion a re-write of the study design was submitted and approved by Battelle's IACUC on 9-26-2008 and ACURO on 10-3-2008. The revised 773 protocol was initiated on October 27, 2008. The revised design tested 4 dose levels of each of 3 NSAIDs (full mouse equivalent dose, ½, ¼, and 1/8; except for 4MP which is ½ mouse equivalent dose model, ¼, 1/8, and 1/16) with and without clobetasol and with or without bandaging these sites. Each pig had one of four sites treated with a dose of NSAID only, 1 site with NSAID plus Clobetasol (a steroid), one site of clobetasol only and the last site of no treatment. Assessments were conducted at 48 h, 72 h, and 7 days and include IR images, photographs, clinical assessments (size, modified Draize and edema), and reflectance colorimetry. Each site was excised on Study Day 7 for histopathology assessment. After conducting the first 3 doses for 4MP and observing continued irritation at all 3 dose levels, consultation with the technical point of contact determined that 1/32 of the full mouse equivalent dose should be applied instead of the 1/16 dose as determined. The *in vivo* portion of 773 was completed on Dec. 24, 2008. Data is in for QA review with some binders completed and the data submitted to the Statistics department. Preliminary Statistics on histopathology (data has been peer reviewed and in QA) and some of the clinical parameters has indicated that OHV with clobetasol and Capsaicin with clobetasol at the highest doses administered were best at ameliorating the effects of HD. OHV was only slightly better than CAP. A draft final report was initiated in mid January 2009 and is nearing completion.

Task 10 was expanded to design, fabricate, and perform dose ranging studies using a chlorine vapor exposure system to produce superficial and deep dermal injuries on the ventral abdomen of weanling swine. In addition, a non-GLP efficacy study was conducted to optimize a treatment regimen using either Capsaicin or Diclofenac with clobetasol that will ameliorate the effects of HD and to select the best therapeutic regimen to use in a future GLP study.

7. A draft final report is being completed. Summary: The chlorine vapor Protocol 910 was approved by Battelle's IACUC on February 19, 2009 and USAMRMC's ACURO approval was received on March 13, 2009. The chlorine vapor exposure system for Study 910 was designed, fabricated and characterized. The study began on May 6, 2009, using 26 dose-ranging animals to determine the concentration and exposure time of chlorine vapor that will induce a superficial dermal injury and a deep dermal injury in the weanling swine model. The first three animals were

exposed to chlorine concentrations of 1 g/L, 1.6 g/L and 2.9 g/L (pure chlorine) for 5, 15, or 30 min in a dry environment with no apparent lesions created. The fourth animal had exposure sites hydrated with distilled water for either 5 or 15 min immediately prior to the 2.9 g/L pure chlorine exposures with no apparent lesions. The client directed that Animals 5 and 6 have 2 sites covered with a 5×5 cm Teflon sheet with an 8 mm hole in the center and affixed with double-sided carpet tape to the rim of the vapor cups. The opposite 2 sites had a 5×5 cm square of cotton t-shirt fabric wetted with simulated sweat and placed under the vapor cups for a 10 min pure chlorine exposure. Only inconsistent, non-uniform superficial dermal lesions were visually apparent and no evaluations or tissues for histopathology were taken. The next 2 animals incorporated a 15 min pre-exposure to a simulated sweat soaked t-shirt fabric positioned on two midline opposite sites (e.g., A and B) with a 400 g weight on top, then re-wetted with simulated sweat prior to two adjacent site exposures of 10 (e.g., sites A and C) and 20 min (e.g., sites B and D) of 2.9 g/L chlorine vapor. The two posterior sites (e.g. C and D) were wetted with simulated sweat just prior to the 10 and 20 min 2.9g/l chlorine vapor exposures. Tissues for histopathology were taken but only photographs and lesion size with descriptions were evaluated at 24 and 48 h. These lesions were more uniform and appeared to be very superficial dermal lesions. Animals 9 and 10 compared simulated sweat on t-shirt fabric and on a disk of No. 2 Whatman filter paper under the vapor cups during 2.9 g/L chlorine exposures of 30 and 45 min. Two more animals were exposed using simulated sweat on filter paper disks for 60, 75 and 90 min exposures, resulting in only superficial dermal lesions (based on histopathology results). In discussions with the client, it has been determined that to create lesions a wetted material (fabric or filter paper) must be applied to the skin for at least 15 min before exposing the skin surface to 2.9 g/L pure chlorine vapor exposure for at least 30 min to result in a superficial dermal injury. After the 30 min exposure the fabric and/or filter paper was dry. It does not matter whether fabric or filter paper is used to apply simulated sweat to the skin surface. It is also clear that increasing the exposure time beyond 30 min slightly increased the depth of injury (histopathology results), but not beyond a superficial dermal lesion. Thus a deep dermal injury by this technique was not created. A tentative conclusion is that a dry 2.9 g/L pure chlorine vapor stream requires moisture to create a lesion and the depth of injury is determined by maintaining a constant source of moisture. The system was configured with a nafion tube to deliver humidified chlorine vapor. Pure chlorine vapor at 2.9 g/L with 75% relative humidity was used to expose two ventral abdominal sites for 31 min and two for 60 min without wet filter paper used. The lesions were observed to be superficial dermal in depth and not uniform across the site. Three animals were exposed for 30 min to humidified chlorine vapor and ~ 0.5 mL simulated sweat soaked filter paper applied to all 4 sites. Although very corrosive to the exposure system itself, histopathology again confirmed that only superficial dermal lesions could be created with the humidified chlorine vapor system, and these were inconsistent in severity. The system was reconfigured to deliver the saturated dry chlorine vapor (2.9 g/L chlorine) in a dry environment. A weighed filter paper was soaked with simulated sweat, reweighed and placed on each of four ventral abdominal sites on three animals, then exposed for either 3, 6, 10, or 15 min to determine when dryness occurred by reweighing the filter paper after the exposure and calculating the difference. The next four animals were exposed for various exposure lengths (10 to 30 min) while the simulated sweat was applied every 3 min to maintain the wetness of the filter paper. Histopathology again verified that only superficial dermal lesions were created.

In consultation with the sponsor it was noted that it was not possible during the course of these experiments to generate a deep chlorine burn (with or without humidified chlorine vapor). Therefore, the sponsor authorized moving onto the genomics portion of the study using a total of 12 animals targeting four early time-points post exposure (i.e., 1.5, 3, 6, and 24-hr) for clinical

pathology, ELISAs for detecting inflammatory biomarkers in blood, and toxicogenomic analyses to examine underlying molecular mechanisms of tissue damage, identify biomarkers, and identify potential therapeutic targets. These genomics animals were exposed exactly like the validated superficial dermal model animals – that is, each site was exposed to 2.9 g/L of pure dry chlorine for 30 min onto 2-mL sweat-saturated filter paper disks with no rewetting. In-life studies were completed mid-December 2009. All study binders are through QA. Report writing is in progress and awaiting finalization of pathology, statistics, chlorine vapor system and genomics report appendices.

8. A draft final report is being completed. Summary: Protocol 911 was approved by Battelle's IACUC on March 9, 2009 followed by ACURO on March 31, 2009. Superficial dermal and deep dermal lesions were created using the sulfur mustard weanling swine model. This study is designed to optimize the treatment regimen (onset of treatment, number of treatments per day, and the duration of the treatment) using the down-selected dose of the selected NSAIDs with clobetasol. The test articles are two NSAIDs, Capsaicin (Zostrix HP 0.075%) and diclofenac (Solaraze 3%), each applied topically followed by clobetasol propionate 0.05%. Lesions are evaluated at 24 and 48 h, and at 7 d by clinical observations (including Modified Draize scoring), wound measurements, photographs, and non-invasive bioengineering methods (reflectance colorimetry, evaporimetry and infrared thermography), and histopathology. The study was initiated June 1, 2009, and the *in vivo* portion completed early October. Most data, except histopathology are through QA review. Histopathology and peer-review are 100% complete. All data have been submitted to the statistics unit to for analyses. Descriptive statistics and bar graphs of the data are being reviewed and the ANOVA analyses are being performed. A draft pathology report has been initiated and is in peer review. A draft of the final report has begun and is waiting for pathology and statistical data.

Task 10 was expanded (Year 4) to conduct a definitive therapeutic efficacy study with the selected treatment regimen and treatment tested in Year 3 under Protocol 911 against sulfur mustard under GLP regulations (Protocol 1054). A second study , non-GLP, will test additional therapeutic compounds along with the optimal NSAID and steroid identified in Protocol 911 against sulfur mustard lesions and chlorine lesions.

9. *In vivo* portion completed and data are in review, QC and statistical analysis process. Histopathological tissues are being processed and prepared for reading by a board certified pathologist. Summary: A definitive therapeutic efficacy study with the selected treatment regimen and treatment tested in Year 3 under Protocol 911 against sulfur mustard under GLP regulations (Protocol 1054) was initiated in April 2010. The selected NSAID was Diclofenac Sodium 3% with clobetasol. The treatment onset was at 2 hours post-exposure with treatments occurring twice daily for 5 days. The in-life phase was completed May 3, 2010. Data is currently undergoing QC/technical reviews. Skin tissues are being processed for histopathology and immunohistochemistry staining.

10. One compound of two to be tested in Phase I of Protocol 1055 *in vivo* portion is complete and the second compound is scheduled in early June 2010. Phase II will follow the second compound. Summary: Protocol 1055 has been written in two parts, Phase I will test two additional therapeutic compounds with the optimal treatment and treatment regimen from protocol 911 and test the efficacy of the new therapeutic compound(s) as a single treatment against HD induced injuries. Phase II will test a treatment and treatment regimen against chlorine vapor injuries. Protocol 1055 Phase I was initiated April 2010. Animals are being treated with Subcutaneous

Enbrel and treated topically as follows: one site clobetasol, one site diclofenac, one site clobetasol + diclofenac, and one site untreated. The second drug (thalomid) has been selected for testing and will be administered orally with topical treatments as outlined above. A third group will not receive systemically administered drugs and will only be treated topically as outlined above. Phase II testing (CLV) will be scheduled for June following the conclusion of phase I.

Task Order 0011 - Definitive Animal Studies with Midazolam

Study Director(s): Dr. Michael Babin

Government POC(s): Mr. Andrew Atkinson / Dr. John McDonough (CBMS)

Period of Performance: September 20, 2006 through November 30, 2010
(Research ends July 30, 2010)

Progress:

657-G823411 and 785-G823411 Studies

The 657-G823411 guinea pig efficacy testing of midazolam against RVX challenges was initiated on April 13, 2009 and completed in May 2009. The evaluation of the EEGs for the 657 study continued during May and June and was completed in July of 2009. Data for the 657-G823411 was sent to Quality Assurance and a draft final report was initiated.

The analysis of the diazepam plasma samples for 785-G823411 was completed in May. Battelle Columbus received the first of five shipments of rhesus monkeys (21 animals) from NIH on June 3, 2009 for quarantine and surgery at Battelle, King Avenue. The second of five shipments of rhesus monkeys (21 animals) was received from NIH on June 3, 2009 for quarantine and surgery. The third of five shipments of rhesus monkeys (21 animals) from NIH was received on July 21, 2009 for quarantine and surgery at Battelle, King Avenue. Telemetry surgeries on the first group of 21 monkeys was initiated on July 7, 2009 and completed on July 17, 2009. Telemetry surgeries on the second group of 21 monkeys was initiated on July 22, 2009 and completed on July 31, 2009. Dr. John McDonough attended a meeting concerning primate EEG analysis held at the BBRC on July 21 and July 22, 2009. Mr. Andy Atkinson and Dr. Doug Reichard attended the meeting on July 22, 2009. The BBRC received the first group of 21 monkeys with telemetry implants from Battelle Columbus on July 27, 2009 for the start of GF challenges on August 6, 2009. Battelle Columbus received the fourth of five shipments of rhesus monkeys (21 animals) from NIH on August 11, 2009 for quarantine and surgery at Battelle, King Avenue. BBRC received the second group of 21 monkeys with telemetry implants from Battelle Columbus on August 13, 2009 for the continuation of the GF challenges. Telemetry surgeries on the third group of 21 monkeys were initiated on August 27, 2009. Battelle Columbus received the fourth of five shipments of rhesus monkeys (21 animals) from NIH on August 11, 2009 for quarantine and surgery at Battelle, King Avenue. BBRC received the second group of 21 monkeys with telemetry implants from Battelle Columbus on August 13, 2009 for the continuation of the GF challenges. Telemetry surgeries on the third group of 21 monkeys were initiated on August 27, 2009. Completed GF challenges on September 28, 2009 and initiated the VX challenges on October 1, 2009. Amendment 2 generated regarding change to primate EEG evaluation and sent out for review. BBRC received the fourth group of 21 monkeys with telemetry implants from Battelle Columbus on October 5, 2009 for the continuation of the agent

challenges. Telemetry surgeries on the last group of 20 monkeys were initiated on October 15, 2009 for use in the ongoing VX challenges.

A 657-G823411 draft final report was sent to the sponsor on January 5, 2010, for comments. Comments from the sponsor on the draft final report were received on January 29, 2010

The primate challenges with VX challenges continued throughout November. In November, Protocol Amendment 2 was generated regarding change to primate EEG evaluation and sent out to the sponsor for review. The BBRC received the final group of monkeys with telemetry implants from Battelle Columbus on November 9, 2009, for the continuation of VX-agent challenges. The VX challenges in primates were completed in December 2009. Protocol 785 Amendment 2 regarding change to primate EEG evaluation was signed and registered in December. The pathology on the VX-challenge animals continued throughout December and was completed in January of 2010. The EEG analysis for the 785-G823411 GF and VX challenges was completed in February 2010.

The draft final report for the method development study 678-G823411 was submitted to CBMS for review and to Quality Assurance (QA) for audit on February 26, 2010. The draft final report for the cholinesterase conformation study 755-G823411 was submitted to CBMS for review and to QA for audit on February 26, 2010. The draft final report for the 657-G823411guinea pig midazolam efficacy study was submitted to CBMS for review and to QA for audit on March 1, 2010. The draft final report for the 785-G823411 NHP midazolam efficacy study was submitted to CBMS for review and to QA for audit on March 9, 2010.After receiving comments from the sponsor; the 755-G823411 final report was completed and submitted on April 12, 2010. The 678-G823411 draft final report comments were received on April 22, 2010 and the report finalized except for signatures. The study binders and reports are completed, audited and are ready for archival. The Final Reports will be signed when CBMS determines the format of the final report required for FDA submission. After addressing comments from the sponsor, updated draft final reports for 785-G823411 and 657-G823411 were submitted to CBMS for review on April 28 and April 30, 2010 respectively.

892-G823411 Guinea Pig PK Studies

In the first quarter of 2009: The in-life portion of the 892 pilot study, plasma midazolam analysis, and PK analysis of the data was completed. The results of the pilot study indicated that challenge with GB and treatments did not affect the PK parameters. The PK report for this study was completed. The overall report for this study was initiated this quarter.

In the second quarter of 2009: The report underwent QC and QAU review and the final formatting of the draft final report was in progress.

In the third quarter of 2009: The draft final report for Study 892 was submitted to CBMS (November 6, 2009).

In the fourth quarter of 2009: Editorial comments were received from CBMS (April 20, 2010). Corrections to these comments will be incorporated in early May, 2010. The 892 Final Report will be signed when CBMS determines the format of the final report required for FDA submission.

893-G823411 Guinea Pig PK Studies

In the first quarter of 2009: The in-life portion of the definitive PK study and analysis of plasma sample for midazolam was completed except for a few dose levels that needed to be repeated. On one day of the study this was due to the dose level being four-fold lower than expected. Other repeats were due to missing several blood sample due to failure of the Culex to draw a blood sample. This could be because of animal's position, and is difficult to prevent on a small percentage of animals.

In the second quarter of 2009: Several midazolam dose levels were repeated (August 28 and 31, 2009) due to mis-dosing or failure of blood sampling by the Culex. A second repeat dosing was carried out on October 26, 2009 after approval of Amendment #4 by the IACUC and ACURO. The initiation of the report was his quarter and it was written in parallel with completion of the experiments in order to expedite the report completion.

In the third quarter of 2009: The In-Life portion of this study was completed (November 2009). Plasma midazolam data was sent to QAU for review. In December, 2009, QAU completed the audit of the plasma midazolam data which was then followed by PK analysis. The PK analysis was completed in December 2009, and the PK report audit was initiated. In January, 2010, the PK data was submitted for statistical analysis.

In the fourth quarter of 2009: The 893-G823411 draft final report was submitted to CBMS (February 4, 2010). Comments were received from CBMS on March 10, 2010 and changes incorporated. The study binders and report were completed, audited, and are ready for archival. The 893 Final Report will be signed when CBMS determines the format of the final report required for FDA submission.

Task Order 0012 - Studies to Support the U.S. Army Medical Research Institute of Chemical Defense Collaborative Research Facility (CRF)

Program Manager: Rebecca McKee, B.S.

Government POC(s): CPT (Dr.) Patrick Everley (MRMC-USAMRICD)

Period of Performance: September 25, 2006 through July 20, 2011
(Research ends July 06, 2011)

Progress:

The Collaborative Research Facility (CRF) has continued as a functioning laboratory, routinely running *in vivo* and *in vitro* studies. Support of Task 12 includes both scientific support of studies and laboratory maintenance, as well as programmatic and facility support.

- General activities for the CRF and Task 12 included:
  - Hosted tours for a variety of groups (Government, foreign groups, etc.)
  - Prepared labs for Ribbon Cutting
  - Continued communicating with collaborators about current and future studies
  - Brought two more animal rooms on-line
  - Completed Room 4 hood installation and certification

- Prepare the CRF for flooring repairs and perforated ceiling installation in labs 14 and 16
- Returned laboratories to working order after floor and ceiling repairs were completed
- Began Annual Equipment Inventory
- Track training records for CRF personnel
- Assemble spreadsheet to enable tracking of AUP Amendments
- Update spreadsheet of AUP Amendments
- Maintained Chemical Inventory List and disposed of expired chemicals
- Maintained Master Schedule
- Completed Task 12 quarterly reports and semi-annual reviews
- Updated Intranet SharePoint Site
- Work to provide access to Extranet for outside collaborators
- Update Extranet SharePoint Site
  - Created site for – Dr. Gordon, Dr. Long, Dr. Chilikuri, Dr. Garcia, Dr. Saxena, Dr. Nambiar, PMC and Dr. diTargiani
- Prepare and process purchase requests for necessary laboratory equipment
- All Battelle personnel started process for CAC renewals
- Weekly attendance at Waters Nugenesis SDMS (Scientific Data Management System) Teleconference Planning Meetings (first quarter only)
- Waters Nugenesis SDMS installation completed
- Provide input for and attend quarterly Research Division Meetings
- Performed agent freezer inventory
- Attended all required training
- Maintained laboratory notebooks
- Performed sink and eyewash checks
- Two CRF personnel attended the 2010 SOT Meeting in Salt Lake City, UT
- Received and unpacked supplies
- Attend weekly CRF staff meetings
- 9 AUPs in process or completed
- 2 SOPs completed and submitted to the QA office for approval

- Ongoing and Completed CRF Studies:
  - Premiere Micronutrient Corporation (PMC)
    1. Working closely with Dana Anderson to solve the issue of the rats dying during intubation.
    2. Arranged to have rats Pretreated in the CRF and exposed in 3100.
    3. Successfully exposed the last 16 rats with no deaths due to intubation.
    4. Completed 6 PMC studies
       - Gavaged animals, intubated and exposed to HD
       - At 4 and 24 hours, blood was drawn and lungs harvested and fixed
       - Prepared and shipped PMC Samples to collaborator.
    5. Completed first stage
  - Chilikuri
    1. Completed 6 Enzyme studies for Dr. Chilukuri.
       - Pretreated mice with enzymes
       - Collected blood samples to determine enzyme levels
       - Challenged with nerve agents.
    2. Initiated new amended protocol

- Nambiar
    1. Method for analyzing GD was developed
    2. The Nambiar agent dilution was verified
    3. Completed 23 studies
        - Animals were injected IM with various anti-seizure medications after exposure to nerve agents
        - The animals are monitored for 24 hours for seizure activity
        - Blood was collected and various organs removed for further testing
        - EEG transmitter sets were removed from each animal and returned for reimplantation or returned for refurbishment.
- diTargiani Enzyme experiment
    1. Human BChE inhibited with GD and VX.
    2. Characterized the hydrolysis of G type nerve agents by prolidase Pon1, OPAA, OPH, and SMP30.
    3. Completed 11 studies for Dr. DiTargiani to obtain rate constants of the hydrolysis of nerve agent by catalytic bioscavengers
    4. Conjugates of AChE and BChE were made for reactivation studies performed at WRAIR.

Task Order 0013 - Evaluation of a Bioscavenger in a Nonhuman Primate Model of Chemical Agent Poisoning

Study Director(s): Dr. Patrick Sabourin / Mr. Mark Perry

Government POC(s): Dr. Todd Myers (MRMC-USAMRICD)

Period of Performance: April 20, 2007 through November 30, 2010
(Research ends June 30, 2010)

Progress:

In the first quarter of 2009: The Task 2, Scavenger Efficiency Study was initiated. On May 18, 2009, eight cynomolgus macaques (NHPs) were received from USAMRICD. Neurobehavioral testing was initiated on May 19, 2009. From May 19 to July 10, 2009 animals were quarantined, acclimated to conditions of handling and treatment and sham exposed. During this period, NHPs were tested on neurobehavioral panels twice per day except weekends and Holidays. The temporal response differentiation (TRD) and delayed match-to-sample (DMTS) neurobehavioral tasks were utilized to evaluate cognitive, sensory, and motor function. Each animal received 4 days of sham treatment with two of these including blood collections at 1, 4, and 24 hr post-Bioscavenger injection since these bleeds just prior to testing could affect testing performance. The sham treatments included mock Bioscavenger injection (Bioscavenger replaced with saline), and 24 hr later, putting the NHP in the exposure chair for 1 hr in the hood with fresh air exposure, washing the NHP's head with water to simulate decontamination, allowing the NHP to sit in chair in the lab during room monitoring, and then returning the NHP to its home cage.

In July, the eight NHPs were injected with Bioscavenger and, 1 day later, challenged with 2× LD_{50} soman (GD). Following challenge, animals were decontaminated with RSDL and returned to their home cages for testing. Blood samples were collected at approximately pre-dose, 1, 4, 7, 24, 32, 56, 104, 176, 344,

and 512 hr post Bioscavenger dosing. These samples were shipped to USAMRICD in August, 2009 for cholinesterase analysis.

No animals succumbed to GD challenge. Clinical observations on challenged animals included miosis in all animals within 5-8 min after initiation of the challenge. In some animals very slight tremors, or uncoordination in movements were also noted; however, these effects were very slight and almost imperceptible.

In the second quarter of 2009: The blood sampling and neurobehavioral testing continued for animals pre-treated with Bioscavenger and then challenged with GD. The last 2 weeks of August the positive control animals were challenged with GD; each of these animals was "sham" pre-treated with saline. Following GD challenge, each NHP was tested on neurobehavioral panels twice per day for 21 days. Blood samples were collected at approximately pre-dose, 1, 4, 7, 24, 32, 56, 104, 176, 344 and 512 hr post saline (sham) dosing. These samples were shipped to USAMRICD for cholinesterase analysis.

None of the non-Bioscavenger treated animals succumbed to GD challenge, however, mild to serious clinical signs of organophosphate poisoning were observed in most of these animals. Clinical signs of challenged animals included miosis in all animals within 5-8 min after initiation of the challenge. Other signs included labored breathing, convulsions and prostration. All animals recovered based on clinical observations.

A draft final report, minus the neurobehavioral results and conclusions was submitted to Dr. Todd Myers in October, 2009.

In the third quarter of 2009: The results of this study were presented as a Poster at the CBD S&T Meeting in Dallas, TX in November 2009. The Poster was entitled "Evaluation of the Efficacy of Bioscavenger against Inhaled Soman using Neurobehavioral Function Tests in Cynomolgus Macaques" and was authored by Patrick J. Sabourin, Amiee E. Sivillo, Amanda H Jellick, William E. Hart, Pamela H. Olson, Vanessa L. Little, Todd M. Myers, and Andrew J. Bonvillain. In December, 2009, P. Sabourin and T. Myers discussed the format of the final report and the final report was written in December 2009 and January 2010.

In the fourth quarter of 2009: The results of this study were presented as a Poster at the Society of Toxicology Meeting in Salt Lake City, UT in March, 2010. The Poster was entitled "Efficacy of Bioscavenger against Inhaled Soman using Neurobehavioral Function Tests in Cynomolgus Macaques." and was authored by Amiee E. Sivillo, Patrick J. Sabourin, Mark R. Perry, Todd M. Myers, and Andrew J. Bonvillain. The draft final report was submitted to Dr. Todd Myers on March 16, 2010 and the final report was submitted to Dr. Myers on April 15, 2010. Slides were prepared for an oral presentation at the BioScience Review Meeting to be held in Hunt Valley, MD in May, 2010.

Task Order 0018 – Collection of Tissue Samples from Testing Compounds Against Vapor-Induced Sulfur Mustard Injury in the Hairless Mouse Model

Study Director: Aimée E. Sivillo

Government POC(s): Dr. David Lenz and Dr. John Graham

Period of Performance: September 22, 2009 through September 21, 2010
(Research ended April 26, 2010…report in progress)

Progress:

This task order was approved on September 22, 2009. The euthymic hairless mouse model (CRL:SKH1-hr) will be used to assess inflammatory and systemic effects of potential therapeutic countermeasures against vapor induced sulfur mustard (HD) injury.

IACUC approval was obtained on 11/20/09 and ACURO approval was obtained on 12/3/09. The study protocol was signed and the study was initiated on 12/10/09. We performed a preliminary POD (proof of decontamination) study using 6 mice. Animals were exposed to HD vapors for 6 minutes (maximum planned for study). Following one hour of "off-gassing" and proper procedures for processing, samples were taken to determine contamination potential after the one-hour time period. It was determined through this study that animals can be safely removed from engineering controls one hour following vapor exposure.

A dose-ranging study was conducted on 1/12/10 at various vapor exposure lengths. At Days 1, 3, and 7 following exposure, we harvested skin tissues from animals exposed at all vapor exposure lengths to ultimately select an optimal length which induced uniform, superficial dermal (second degree) injuries for efficacy studies. The length of exposure as determined by the dose-ranging study and further fine-tuning of the model was 6-minutes.

Six potential therapeutic countermeasures to injury were tested for efficacy in ameliorating HD-induced pathology. The first 2 compounds were tested following (once daily for 6 days following) a 3/2/10 challenge with HD vapor. The last 4 compounds were tested following (twice daily for 6 days following) a 4/12/10 challenge with HD vapor. Each of these studies went out for 14 days. At Days 1, 3, 7, and 14, assigned animals were photographed, assigned a draize score, and euthanized for tissue harvesting. All tissues were sent to Rutgers for histopathology analysis.

The draft letter report is in progress.

Task Order 0019 - Treatment of Sulfur Mustard Exposure using the Rabbit Eye Model.
Use of Standard Ocular Pathology Measurements and Molecular Response Methods of Proteomics and Metabonomics to Assess Pathology Level and Treatment Success Response

Study Director(s): Dr. Michael Babin

Government POC(s): Dr. John Schlager (DTRA-AFRL)

Period of Performance: August 10, 2007 through November 30, 2010
(Includes Submission of Final Report)

Progress:

The in-life Phase 2 Antibiotic and Corticosteroid Treatment Efficacy study under Protocol 750 was completed on June 10, 2008. Corneal tissue harvested from rabbits on Harvest Days 42 and 63 were shipped to the sponsor and sponsor representative for evaluation. Data was collated and neovascularization scored for statistical evaluation.

On October 9, 2008, Dr. John Schlager, Mitchell Meade and Pavel Shiyanov attended a briefing at the BBRC on the progress of the 750-G823419 study. Data was presented from the Phase 1 and Phase 2 animal studies and the preliminary statistical report generated by Battelle on the Phase 2 results. A presentation was also given on data from the proteomics studies being conducted at the Air Force Research Laboratory. After review of the data, a decision was made to accept the model generated in Phase 2 for use in the Phase 3 anti-neovascularization study. The challenge dose chosen was a 0.4 µL dose of neat sulfur mustard. The treatments will consist of Polymyxin B Sulfate and Trimethoprim Ophthalmic Solution for the antibiotic and Prednisolone Acetate Ophthalmic Suspension 1% for the anti-inflammatory drug. After review of the data, Dr. Robert Enzenauer selected three drugs to be used in the upcoming Phase 3 study: methotrexate, cyclosporine, and Avastin. Phase 3 studies were tentatively scheduled for January of 2009.

On January 27, 2009 a meeting was held with Dr. John Schlager at WPAFB to discuss issues regarding Phase 3 of the 750-G823419 study. The formulation of drugs for the Phase 3 study was discussed and the study planned for March 2009.

Upon recommendations by the study ophthalmologist, Dr. Robert Enzenauer, the sponsor agreed to test the following anti-neovascularization drugs in the Phase 3 study: Bevacizumab, cyclosporine and Methotrexate. Amendment 4 was prepared allowing for an additional control group and the revised study plan. The amendment was approved by Battelle IACUC on February 11, 2009 and by the U.S. Army Animal Care and Use Review Office (ACURO) on February 26, 2009. The drug formulations were prepared by Central Ohio Compounding Pharmacy, Columbus, Ohio. Rabbits were ordered and arrived at Battelle on March 10, 2009 for use on study. Forty rabbits were challenged on March 25, 2009 and treatments successfully administered. Clinical assessments were conducted on Study Days 7, 21, 42, 84, and 112. Following the last assessment on Study Day 112, corneal tissue and aqueous humor was harvested and shipped to AFRL for histopathology and protein analysis.
On December 16, 2009, a meeting was held at Wright Patterson Air Force Base with the sponsor to discuss the 750-G823419 study data and to plan the upcoming Phase 4 study. As a result of the discussions, a decision was made to modify the existing Phase 4 study. Based on the review of the Phase 3 data, drugs were selected for use on the modified Phase 4 study. On January 15, 2010, Amendment 5, containing the updated study design was submitted to the sponsor for review. Amendment 5 for the updated 750-G823419 Phase 4 study was submitted to the Battelle IACUC on February 12, 2010 for review and received approval on February 15, 2010. The Amendment was submitted to the ACURO on February 15, 2010 for review and received approval on February 18, 2010. Arrangements for the formulation of the test materials were made with Central Ohio Compounding Pharmacy in Columbus, OH. The study was designed for two challenge days. Challenge Day A rabbits arrived at the BBRC for quarantine on April 13, 2010. Pre-challenge eye assessments were conducted on challenge Day A animals on April 19, 2010. Challenge Day B rabbits arrived at the BBRC for quarantine on April 20, 2010. The test material for the Challenge Day A rabbits were delivery to the BBRC by Central Ohio Compounding Pharmacy on April 22, 2010. Sulfur mustard challenges on the challenge Day A rabbits was performed on April 23, 2010. Study Day 3 assessments were conducted on April 26 and the Study Day 7 assessments and tissue harvest were conducted on April 30, 2010. Challenge Day B rabbits were scheduled for sulfur mustard challenge on May 7, 2010.

Preparation of the 750-G823419 final report was started. Phase 4 study is planned for the following quarter.

Task Order 0020 - Personnel Decon: Live Chemical Agents Protocol

Study Director(s): Mr. Thomas H. Snider

Government POC(s): Dr. Dr. Ernest Braue (MRMC-USAMRICD)

Period of Performance: September 15, 2008 through December 31, 2009
(Research ended; Final Report Submitted December 17, 2009)

The objectives of this task were to

- determine the degree of offgassing of thioanisole, sulfur mustard (HD), and VX detected in head space air from samples of Steris MultiPurpose Wipe (MPW) dosed with 5 µL of those compounds,
- determine the efficacy of each of four decontaminants (MPW, RSDL, M291 Skin Decontamination Kit, and soapy water) relative to nothing against a 5-µL dose of thioanisole, HD, or VX applied on cryopreserved porcine skin,
- determine the lesion area ratio (LAR) for each of the four decontaminants in New Zealand White rabbits challenged topically with HD.
- determine the protective ratio (PR) for each of the four decontaminants in Hartley guinea pigs challenged topically with VX.

Progress:

- Further testing of MPWs against a sulfur mustard challenge was requested and possible with no increase in task funding. The difference in this test was that the MPWs were rolled against the test site skin. This two-day test has been conducted, and MPWs were found to offer no statistically significant decontamination effect in terms of reducing lesion areas of erythema.
- A Final Report was submitted on 17 December 2009. All costs have been recovered, and this task has been closed.

Task Order 0024 - Post-Exposure Medical Countermeasures Against Inhalation Toxicity of OP

Principal Study Investigator: Aimée E. Sivillo/Dr. Michael C. Babin

Government POC(s): Dr. Madhusoodana Nambiar (DTRA/WRAIR)

Period of Performance: September 21, 2009 through July 20, 2011
(Research ends February 20, 2011)

Progress:

The microinstillation guinea pig model for this study will be used to assess various Medical Countermeasures (MC) against OP toxicity. This task order was approved on September 21, 2009.

IACUC approval was obtained on 11/13/09 and ACURO approval was obtained on 12/3/09. The study protocol was signed on 12/17/09. The study was initiated with training procedures performed on study-specific animals. The client visited to bring aerosol equipment and further discuss details of the study. Once training for the study is completed, a safety study will take place to confirm that all procedures on study are safe to perform. This will be followed up with a MLD study to determine appropriate dose for efficacy studies. Further studies will begin at that point. Each study will test 3 MC compounds for efficacy against toxicity of OP exposure through inhalation. A total 4 studies (4 days each study) will be performed, resulting in up to 12 MC compounds tested.

The client has visited on a number of occasions. Because processes are understood, equipment is available, and hood space in the lab is available, the current plan is to perform the safety study in May, followed up by the MLD beginning in August. Formal training is still in progress.

Task Order 0028 - Comparative Efficacy of Intravenous Hydroxocobalamin and Sodium Thiosulfate/Sodium Nitrite Administrations Following Intravenous Poisoning with Potassium Cyanide in Adult Male Beagle Dogs

Task Leader: Dr. Frances Reid / Dr. Brian Roche

Government POC(s): Dr. Gary Rockwood (USAMRICD)

Period of Performance: April 22, 2009 through August 21, 2010
(Research ends April 21, 2010)

Progress:

The Task was awarded April 22, 2009 and work authorization received. Draft protocols received Battelle IACUC approval on July 20, 2009 (Experiment 2A) and July 23, 2009 (Experiments 1 and 2B), respectively. The three protocols were submitted to the USAMRMC ACURO office and approval received August 26, 2009. The original company used in the Boron et al. study to perform cyanide analysis and hydroxocobalamine analysis of samples was subcontracted and informed Battelle that they were not available to do the analyses. Michigan State Diagnostic Laboratory was subcontracted to conduct the cyanide assays and the hydroxocobalamine assay was set-up in-house. This delayed the start of the first experiment, Protocol G823428-DOSE, and after consultation with the sponsor the Median Lethal Dose (MLD, Experiment 2A, Protocol G823428-MLD) study was conducted first. The MLD 2A study began in September 9, 2009 and completed on September 29, 2009. After evaluation of seven animals in an up-down design the primary end point indicated a dose of 2.78 mg/kg dose of KCN as the MLD in this anesthetized model and will be used in the GLP 2B experiment, Protocol G823428-LETH (LETH). Secondary endpoints have been analyzed and reviewed. A brief study summary is being drafted. Experiment 2B (G823428-LETH) was initiated November 16, 2009 using the 2X the MLD_{50} of 5.56 mg/kg KCN against sodium nitrite with sodium thiosulfate treatment and Hydroxocobalamin treatment. Eleven animals were challenged with 2X MLD50 KCN over a minute by infusion then treated with either IV administration of sodium nitrite and sodium thiosulfate resulting in 3 out of 8 animals surviving, or IV infusion over 7.5 min of Hydroxocobalamin with no surviving animals after Day 2 out of the three studied. The study was stopped in December 2009 as a result of unexpected mortalities and to conduct Experiment 1, G823428-DOSE.

Experiment 1 began on January 11, 2010. Twelve animals were dosed as a physiological model designed to mimic the Borron et al study. In summary, a target concentration of 1.0 mg/mL KCN was infused at a rate of 0.4 mg/kg/min until apnea occurred and continued to be infused for an additional 3 min. KCN infusion was stopped and treatment was initiated (either 150 mg/kg Hydroxocobalamin or 10 mL of a 3% solution of sodium nitrite and 50 mL of a 25% solution of sodium thiosulfate) and ventilation started for 15 min. Twelve animals were completed initially (five animals were vehicle, five animals dosed with sodium nitrite with sodium thiosulfate and 2 animals dosed with Hydroxocobalamin). None of the five vehicle animals survived 24 h, 3 out of 5 sodium nitrite/sodium thiosulfate dosed animals survived and none of the hydroxocobalamin animals survived. These results were contrary to the earlier study (Borron et al) to which this study was to mimic, the study was stopped. Consultation and review of data with Dr. Rockwood identified a difference in concentration of the KCN. Also noted, was a difference in physiological response between the two studies. The Borron study showed an onset of signs within 30 sec as opposed to ~1.5 min in the current study. The Borron study animals responded to KCN more acutely and apnea was observed earlier than the current study. The difference observed was the KCN concentration of 1.1 mg/mL infused at 0.4 mg/kg/min against 0.97 mg/kg at the same infusion rate of the current study. The decision was made to adjust the infusion rate of KCN at the current concentration to mimic the concentration of KCN infused at 0.4 mg/kg/min of the Borron study. A vehicle animal was studied which resulted in a physiological response similar to that of the Borron study. A second vehicle animal was studied with similar results. Consultation with Dr. Rockwood resulted in continuing the study using altered infusion rate to adjust the amount of KCN administered to mimic that which was administered in the Borron study (concentration of 1.1 mg/mL at an infusion rate of 0.4 mg/kg/min). Using this technique, all vehicle dogs (n = 9) died within 24 h (they did not survived the KCN administration), of the sodium nitrite/sodium thiosulfate treated animals eight out of 9 survived 24 h with 6 out of 9 surviving 14 days (n = 9), and of the Hydroxocobalamin treated animals eight out of 9 survived 24 h with 7 out of 9 surviving 14 days (n = 9). This study was completed on February17, 2010. Data has been submitted to statistics and analyses are nearly complete. The draft final report has been initiated.

In April the LETH study was restarted and the initial results indicated the blood cyanide data of the first 11 animals challenged (back in November 2009 on the LETH study) indicated ~3 times the estimated value [n = 11 challenged which resulted in no survival from hydroxocobalamin animals 20 (2 deaths out of n = 2) and sodium nitrite/sodium thiosulfate treated animals showed 3 survivors (3 out of n = 8)]. It was determined that the concentration used to calculate the KCN administration had not been corrected to the new concentration on the dose formulation sheet and the animals were dosed at 3.6 times the MLD of KCN. In November, before the error in concentration was detected, several animals were challenged by 60 s KCN infusion and treated with sodium nitrite/sodium thiosulfate at 5 mL of nitrite over ~2.5 m followed by 10 mL thiosulfate over ~3-5 m, and then complete nitrite followed y thiosulfate by slow infusion. This administration procedure was adjusted based on the response of blood pressure after KCN infusion. This dosing procedure was implemented for the restart of the LETH study in April. Additionally, hydroxocobalamin administration was administered slowly by hand in two animals to determine the cardiovascular effects of increased infusion time and all survived. It was determined that we could use the 7.5 m infusion pump to administer the hydroxocobalamin as originally dosed at the 2 times the KCN MLD. These two treatment techniques are currently being used to complete the 2X KCN MLD at n=30 animals per each treatment. As of May 1, 2010, 21 animals have been challenged. Those treated with nitrite/thiosulfate have nine animals surviving out of 10 treated. The hydroxocobalamin animals have all surviving out of 11 animals challenged.

A proposal for a pilot Cobinamide (novel CN antidote compound) efficacy study (limited dose ranging in dogs and pilot efficacy) was submitted August 31, 2009 and approved September 16, 2009. The protocol to conduct a pilot efficacy study as non GLP was approved by ACURO on March 04, 2010. This study is

anticipated to start early to mid June 2010 after completion of final review of the protocol, final formulation of the test article, and chemistry assay validation.

Task Order 0029 – Characterization of Agent Mechanisms of Toxicity and Toxicokinetic Properties

Study Director(s): Mr. Timothy L. Hayes

Government POC(s): Lt Col Nathan Johnson/Mr. Alfred S. Graziano

Period of Performance: April 24, 2009 through October 23, 2010
(Research ends June 23, 2010)

Progress:

This task was awarded on April 24, 2009. Battelle has contacted the Government to confirm the compounds of interest. A secure Fax was received on April 30, 2009 with a list of the compounds. Names were assigned to the compounds of Interest: Banana, Batman, Wolverine, Spiderman, and Superman. The work is divided into three phases Synthesis, Phase 7, and Phase 8. The Battelle and AFRL/DTRA staff have been communicating by teleconference monthly and was able to meet in person during the Chemical and Biological Defense Science and Technology (CBD S&T) Conference, in Dallas TX, 16-20 November 2009. A summary of progress of each phase of work is provided below.

Synthesis: The synthesis work to produce Banana, Batman, and Wolverine are complete and purification and documentation of purity is complete as well. The synthesis of Spiderman has been challenging. Battelle has been successful in synthesizing the material in small amounts but is currently having problems verifying the purity due to NMR of the suspected impurities not being readily resolved from the desired compound. This work is continuing. The synthesis of the final compound (Superman) is awaiting progress on Spiderman.

Test Plan – Phase 7: The methods development using a 96 well plate ultrafiltration system is being performed. An interference noted with plates previously has been resolved with additional washing/conditioning steps. The optimization of this process has been performed. The screening format using the 96-well plate ultrafiltration versus the single cell ultrafiltration method used in the literature has been successful. The use of a supplier for tissue delivery has been worked out and this work has produced a working SOP. The qualification of the procedure using paraoxon with the tissues has been performed and the data support the use of the method.

Test Plan – Phase 8: Work to optimize the ultra filtrate plate, using various concentrations/dilutions continues. To date the flow-thru is fine with caffeine and anti-pyrene however, neat plasma is a little heavy. The system does yield enough filtrate to support analysis. Work is planned to test warfarin as a control. Due to limited solubility in water it is anticipated that modifiers such as DMSO.

Task Order 0031 - Static Temperature Testing of Reactive Skin Decontamination Lotion (RSDL) with a Time Temperature Indicator (TTI)

Study Director(s): Mr. Timothy L. Hayes

Government POC(s): Dr. Dai K. Liu, MITS-JPMO
Mr David Tally
Mr. Tim Belski, Goldbelt Raven, LLC, MITS-JPMO

Period of Performance: August 20, 2009 through November 19, 2010
(Research ends July 19, 2010)

Progress:

The statistical analysis of the data has been initiated along with the reporting process for the Task. The last two shipments/assays are anticipated to be made in May.

- This task was proposed on May 4, 2009.
- Battelle was awarded the task on August, 20 2009.
- Held teleconference on August 24, with client and BRACCO (Subcontractor) to discuss work plan and protocol development.
- Temptime (TTI manufacturer) provided some examples of the TTI devices and the x-Rite model 404 spectrodensitometer for Battelle to develop technique and train staff on its usage.
- Battelle purchased a newer model x-Rite 528 as a backup to the older model 404 and evaluated it for use.
- Training materials were provided by the Temptime for visual evaluation of the TTIs and written procedures and training was provided to staff for this work.
- Incubator used in the work was qualified using a 12 point Validator 2000 system to assess temperature control and uniformity.
- Protocol has been written, reviewed and approved on January 18, 2010.
- Temptime provided the three lots of Type E TTIs and one lot of Type Z TTIs.
- Started high temperature studies (130 C) with all materials (RSDL (3 lots), Type E (3 lots), and Type Z (1 lot) on January 19, 2010. Optical density and visual measurements were recorded.
- A revised costing was prepared to address additional testing identified in the protocol development.
- Second test day was February 16, 2010 with optical density and visual measurements made per Protocol 1059-G823431.
- Third test day was February 22, 2010 with optical density and visual measurements made per Protocol 1059-G823431. RSDL was shipped to BRACCO and Farrington Lockwood for analysis.

- Fourth test day was March 1, 2010 with optical density and visual measurements made per Protocol 1059-G82343. RSDL was shipped to BRACCO and Farrington Lockwood for analysis.
- RSDL Analysis data from the February 22, 2010 test set was received from BRACCO on March 2, 2010. All packages of RSDL are within specifications.
- A revised costing was prepared to address additional testing identified during the protocol development and submitted to the Government. The Contract modification (Mod 3) to increase the contract by $63,841.00 from $542,355.00 to $606,196.00 was accepted on April 8, 2010.
- Continued to collect data on the following test days: March 15, 22, 29, April 5, 12, 19 and 26.

Plans for May –July l 2010

- Continue testing per Protocol 1059-G823431.
- Perform statistical analyses.
- Draft Final report.

Task Order 0032 – Testing Therapeutic Countermeasures against Ocular Injuries following Sulfur Mustard Exposure in Rabbits

Study Director(s): Dr. Michael Babin/Dr. Jill Harvilchuck

Government POC(s): Dr. Marion Gordon

Period of Performance: September 22, 2009 through June 21, 2010
(Research ends February 21, 2010)

Progress:

Task Order 0032 was proposed on April 17, 2009 and was awarded on September 22, 2009. Study number 1049-G823432 was assigned and a protocol written. The protocol was submitted to the Battelle IACUC for review and approval obtained on November 3, 2009. The approved protocol was submitted to the ACURO on November 4, 2009 and approved on November 6, 2009.

The Study was initiated on November 30, 2009. The in-life portion of the study was completed on December 30, 2009. Tissues were shipped to the sponsor for analysis in December 2009 and January 2010. The initial Quality Assurance audit has been completed on the study and the draft final report is in progress.

Task Order 0033 - Development of a Process to Produce Large Amounts of the Oxime HLo-7

Study Director(s): Eric Lucas, Timothy L. Hayes, B.A.

Government POC(s): Dr. Tsung-Ming (Tony) Shih, USAMRICD

Objective(s): The USAMRICD has a need for large amounts of the oxime HLo-7 which has been made in small quantities but never on a scale in excess of 1-2 grams. Request development of a process to produce this material in greater than 80 gram quantities and to then provide 100 grams of material of >95 percent purity by standard methods such as NMR, IR or elemental analysis.

Period of Performance: March 29, 2010 through December 28, 2010
(Research ends 28 September 2010)

Progress: Task was awarded on March 29, 2010. Staff was assembled and materials have been ordered.

Task Execution Plan (TEP) 0034 – Temperature Testing of Pyridostigmine Bromide (PB)

Study Director(s): Timothy L. Hayes, B.A.

Government POC(s): Dr. David Lenz
Dr Dai K Liu
Mr. David Talley

Status: Mutually agreed postponement between Battelle and the Government until further notice.

KEY RESEARCH ACCOMPLISHMENTS

Task 0001

- N/A

Task 0010

- The efficacy and optimal treatment regimen for the NSAID followed by steroid for treatment of HD- induced superficial and deep dermal lesions was determined from BBRC protocol 911. Report in progress.
- Created a chlorine vapor exposure system and developed a model for superficial dermal injury in swine. Report in progress.
- Completed in-life phase of GLP study for the treatment of HD-induced superficial and deep dermal lesions using diclofenac sodium followed by clobetasol.
- Initiated testing of additional drugs (Enbrel and Thalomid) in conjunction with the diclofenac sodium and clobetasol treatment regimen.

Task 0011

- The 657-G823411 guinea pig efficacy testing of midazolam was completed.
- A 657-G823411 draft final report was sent to the sponsor on January 5, 2010.
- Revised draft final reports for 657-G823411 was submitted to CBMS for review on March 1 and April 30, 2010.
- The agent challenges in the 785-G823411 primates were completed in December 2009.
- Protocol 785 Amendment 2 regarding change to primate EEG evaluation was signed and registered in December.
- The pathology on the VX-challenge animals was completed in January of 2010.
- The EEG analysis for the 785-G823411animals was completed in February 2010.
- The draft final report for 785-G823411 was submitted to CBMS for review on March 9, 2010.
- A revised draft final reports for 785-G823411 was submitted to CBMS for review on April 28, 2010.
- The draft final report for the method development study 678-G823411 was submitted to CBMS for review on February 26, 2010. Comments were received on April 22, 2010 and the report finalized.
- The draft final report for the cholinesterase conformation study 755-G823411 was submitted to CBMS for review on February 26, 2010. After receiving comments from the sponsor; the 755-G823411 final report was completed and submitted on April 12, 2010.
- The Final Reports for 678-G823411 and 755-G823411 will be signed when CBMS determines the format of the final report required for FDA submission.
- The pharmacokinetics (PK) of midazolam in guinea pigs in the presence and absence of GB and treatments and report were completed.

Task 0012

- The Collaborative Research Facility (CRF) is functioning as a laboratory routinely running *in vivo* and *in vitro* studies- 46 studies completed.
- Brought extranet site on-line to allow external collaborators immediate access to experimental data and technical updates.
- Prepared labs for ribbon cutting ceremony.

Task 0013

- The LD_{50} of inhaled GD in cynomolgus macaques was estimated at 9.9 ug/kg body weight with an 87.5% confidence interval of 3.88 µg/kg to18.26 µg/kg. The LCT_{50} was not a good measure of toxicity. The dose-response curve is very steep.
- Based on clinical signs, Bioscavenger clearly protected cynomolgus macaques from the toxic effects of an inhaled lethal dose of GD (2 x LD_{50}), but did not protect against miosis in whole head exposures.
- Bioscavenger was shown to allow for quicker recovery of performance to baseline levels on the neurobehavioral tasks tested (DMTS and TRD) in some but not all of the animals. All animals, treated or positive controls, eventually returned to baseline performance levels.
- It is likely that miosis in both Bioscavenger treated and positive control animals, in some but not all animals, caused decrements in performance for a few days post-exposure.

Task 0018

- Following model development of the hairless mouse model for vapor cap exposure to HD on the back was developed prior to any agent work. A proof of decontamination (POD) study was performed on 12/10/09 to determine if removing hairless mice exposed to HD vapor on backs could be removed from engineering controls after one hour. It was determined that it was safe.
- The Dose Ranging Study HD vapor challenges were performed on 1/12/10. HD vapor exposure on backs was for 1, 2, 3, 4, or 6 minutes. Animals had tissues harvested on Days 1, 3, and 7 following challenges. At each of these time points, photos were taken of lesions (or lack thereof) and Draize scoring was performed at those time points for Day 7 animals. The tissues were evaluated by a pathologist here at Battelle. Based on all data, we decided to move forward with the 6-minute exposure time for the efficacy studies.
- The Compound Efficacy studies were performed beginning with HD vapor challenges on 1/12/10 (2 compounds) and 4/12/10 (4 compounds). HD vapor exposure on backs was for 6 minutes. Animals had tissues harvested on Days 1, 3, 7, and 14 following challenges. At each of these time points, photos were taken of lesions (or lack thereof) and Draize scoring was performed at those time points for Day 14 animals. The tissues were shipped and are being evaluated by a pathologist at Rutgers. The final letter report is in progress.

Task 0019

- Phase 3 studies were started on March 25, 2009. Forty rabbits were challenged on March 25, 2009, treatments were administered and clinical assessments were conducted.
- The Phase 3 corneal tissue and aqueous humor was harvested and shipped to AFRL for histopathology and protein analysis.
- On December 16, 2009, a meeting was held at Wright Patterson Air Force Base to plan the Phase 4 study.

- Phase 4 sulfur mustard challenges on the challenge Day A rabbits was performed on April 23, 2010. Study Day 3 assessments were conducted on April 26 and the Study Day 7 assessments and tissue harvest were conducted on April 30, 2010.
- Challenge Day B rabbits were scheduled for sulfur mustard challenge on May 7, 2010.

Task 0020

- Further testing of MPWs against a sulfur mustard challenge was requested and possible with no increase in task funding. The difference in this test was that the MPWs were rolled against the test site skin. This two-day test has been conducted, and MPWs were found to offer no statistically significant decontamination effect in terms of reducing lesion areas of erythema.
- A Final Report was submitted on 17 December 2009. All costs have been recovered, and this task has been closed.

Task 0024

- To date, model development has been in process (guinea pigs intubated for microinstillation of compound) and processes have been underway. A dry run for the safety portion of the study was performed on 5/17/10 and will be repeated on 6/21/10, resulting from changing processes involving handling of the microinstillation equipment. A safety committee meeting took place on 5/28/10 to review SOP procedures.

Task 0028

- Identified 2.78 mg/kg as the median lethal dose (MLD) of KCN in the anesthetized beagle dog.
- Completed in-life phase of GLP study for head to head comparison of two KCN antidotes against a physiological model of KCN administration.
- Completed in-life for GLP study of assessment for two KCN antidotes against 2 X MLD of KCN (5.56 mg/kg).
- Identified possible modifications of the “prescribed” method of administration of the potential KCN antidotes against doses higher than 2 times the MLD for KCN.

Task 0029

- This task was awarded on April 24, 2009.
- Names were assigned to the compounds of Interest.
- The work is awarded as three phases Synthesis, Phase 7, and Phase 8.
- The Battelle and AFRL/DTRA staff have been communicating by teleconference monthly and was able to meet in person during the Chemical and Biological Defense Science and Technology (CBD S&T) Conference, in Dallas TX, 16-20 November 2009.
- Synthesis: The synthesis work to produce Banana, Batman, and Wolverine are complete and purification and documentation of purity is complete as well.
- Battelle has been successful in synthesizing Spiderman. The purification of the product is problematic.
- Phase 7 Partition Coefficient: The methods development using a 96 well plate ultrafiltration system has been developed and is being performed.
- Phase 8 Protein Binding: The methods development using the ultra filtrate plate has been developed for protein binding and is being performed.

Task 0031

- This task was proposed on May 4, 2009.
- Battelle was awarded the task on August, 20 2009.
- Held teleconference on August 24, with client and BRACCO (Subcontractor) to discuss work plan and protocol development.
- Temptime (TTI manufacturer) provided some examples of the TTI devices and the x-Rite model 404 spectrodensitometer for Battelle to develop technique and train staff on its usage.
- Battelle purchased a newer model x-Rite 528 as a backup to the older model 404 and evaluated it for use.
- Training materials were provided by the Temptime for visual evaluation of the TTIs and written procedures and training was provided to staff for this work.
- Incubator used in the work was qualified using a 12 point Validator 2000 system to assess temperature control and uniformity.
- Protocol has been written, reviewed and approved on January 18, 2010.
- Temptime provided the three lots of Type E TTIs and one lot of Type Z TTIs.
- Started high temperature studies (130 C) with all materials (RSDL (3 lots), Type E (3 lots), and Type Z (1 lot) on January 19, 2010. Optical density and visual measurements were recorded.
- A revised costing was prepared to address additional testing identified in the protocol development.
- Second test day was February 16, 2010 with optical density and visual measurements made per Protocol 1059-G823431.
- Third test day was February 22, 2010 with optical density and visual measurements made per Protocol 1059-G823431. RSDL was shipped to BRACCO and Farrington Lockwood for analysis.
- Fourth test day was March 1, 2010 with optical density and visual measurements made per Protocol 1059-G82343. RSDL was shipped to BRACCO and Farrington Lockwood for analysis.
- RSDL Analysis data from the February 22, 2010 test set was received from BRACCO on March 2, 2010. All packages of RSDL are within specifications.
- A revised costing was prepared to address additional testing identified during the protocol development and submitted to the Government. The Contract modification (Mod 3) to increase the contract by $63,841.00 from $542,355.00 to $606,196.00 was accepted on April 8, 2010.
- Continued to collect data on the following test days: March 15, 22, 29, April 5, 12, 19 and 26.

Task 0032

- Task Order 0032 was awarded on September 22, 2009.
- Study number 1049-G823432 was assigned and a protocol written and approved by Battelle IACUC and the ACURO.
- The Study was initiated on November 30, 2009.
- The in-life portion of the study was completed on December 30, 2009.
- Tissues were shipped to the sponsor for analysis in December 2009 and January 2010.

- The initial Quality Assurance audit has been completed on the study and the draft final report is in progress.

Task 0033

- Task was awarded on March 29, 2010.
- Lab staff were assembled and materials have been ordered.

REPORTABLE OUTCOMES

Not applicable.

CONCLUSION

Not applicable.

REFERENCES

Not applicable.

APPENDICES

Not applicable.

SUPPORTING DATA

Not applicable.